

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 2

In the Specification

Please amend the Specification pursuant to 37 C.F.R. §1.121 as modified by 68 Fed. Reg. 38611 (June 30, 2003) as indicated below.

On page 8, paragraph 2, lines 8-19:

Starch synthases extend regions of α -1, 4 glucan through the transfer of the glucosyl moiety of ADPglucose to the non-reducing end of a pre-existing α -1, 4 glucan. In addition to GBSS, 3 other classes of starch synthase have been identified in plants, SSI (wheat, *Li et al.*, 1999 and GenBank Accession No. U48227; rice, *Baba et al.*, 1993; potato, Genbank Accession No. ~~STSTASNT~~ Y10416, SSII (pea, *Dry et al.* 1992; potato, Edwards et al., 1995; maize, *Harn et al.*, 1998 and GenBank Accession No. U66377) and SSIII (potato, *Abel et al.*, 1996; maize, *Gao et al.*, 1998). In the cereals, the most comprehensively studied species is maize, where in addition to GBSS, cDNAs encoding SSI, and SSIIb have been isolated, and both cDNA and genomic clones for *dull1* have been characterised (*Knight et al.*, 1998; *Harn et al.*, 1998; *Goa et al.*, 1998). In maize, the product of the *dul* gene is known as maize SSII, however this gene is the homologue of potato SSIII.

On page 12, paragraph 2, lines 11-21:

In an alternative embodiment, the isolated nucleic acid molecule of the present invention encodes a wheat starch synthase polypeptide which comprises one or more amino acid sequences selected from the group consisting of:

- (A) GHTVEVILPKY (SEQ ID NO:40);
- (B) HDWSSAPVAWLYKEHY (SEQ ID NO:41);

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 3

- (C) DVPIVGIITRLTAQKG (SEQ ID NO:43);
- (D) NGQVVLLGSA (SEQ ID NO:44);
- (E) AGSDFIIVPSIFEPCLTQLVAMRYGS (SEQ ID NO:45);
- (F) TGGLVDTV (SEQ ID NO:46);
- (G) GIVNGIDNMEWNPEVD (SEQ ID NO:50); and
- (H) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO:53).

On page 14, paragraph 2 (iii), lines 21-27 and page 15, paragraph 1, lines 4-11:

(iii)a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (A) KVGGLGDVVTS (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);
- (E) DVPIVGIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPCLTQLVAMRYGS (SEQ ID NO:45); and
- (H) TGGLVDTV (SEQ ID NO:46).

wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS:2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (A) KTGGLGDVAGA (SEQ ID NO:47);
- (B) GHRVMVVVPRY (SEQ ID NO:48);
- (C) NDWHTALLPVYLKAYY (SEQ ID NO:49);

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 4

- (D) GIVNGIDNMEWNPEVD (SEQ ID NO:50);
- (E) DVPLLGFIGRLDGQKG (SEQ ID NO:51);
- (F) DVQLVMLGTG (SEQ ID NO:52);
- (G) AGADALLMPSRF(E/V) PCGLNQLYAMAYGT (SEQ ID NO:53); and
- (H) VGG(V/L)RDTV (SEQ ID NO:54).

On page 17, paragraph 2, lines 17-20 and paragraph 3, lines 22-31:

Figure 2 (Figure panel 2A-2O) is a copy of a schematic representation comparing the nucleotide sequences of cDNA clones designated wSSIIA (SEQ ID NO:3), wSSIIB (SEQ ID NO:1) and wSSIID (SEQ ID NO:5), encoding the starch synthase II polypeptides from wheat, using the PILEUP programme of Devereaux et al. (1984).

Figure 3 (Figure panels 3A-3G) is a copy of a schematic representation comparing the deduced amino acid sequences of starch synthase II from wheat (wSSIIA (SEQ ID NO:3), wSSIIB (SEQ ID NO:1) and wSSIID (SEQ ID NO:5), maize (maize SSIIa and maize SSIIb; Harn et al., 1998), pea (pea SSII; Dry et al., 1992) and potato (potato SSII; van der Leij et al., 1991). Identical amino acid residues among each of these sequences are indicated below the sequences with "*". The alignments of maize SSIIa with maize SSIIb, and pea SSII and potato SSII are essentially as described in Harn et al. (1998) and Edwards et al. (1995). All sequences are aligned to position the transit peptide cleavage site below the arrow (↓) between residues 59 and 60 of the wSSIIA sequence. The wSSIIP1 sequence, the sequence of SGP-B1 (peptide3), and of eight conserved regions are annotated and underlined.

On page 18, paragraph 4, lines 30-31:

Applicants: . Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 5

Figure 7 (Figure panels 7A-7I) is a schematic representation comparing the deduced amino acid Sequences of the maize (SEQ ID NO:55), potato (SEQ ID NO:56) and wheat SSIII (SEQ ID NO:8) polypeptides.

On page 19, paragraph 2, lines 16-22:

Figure 9 (Figure panels 9A-9F) is a schematic representation showing the position of conserved amino acid sequences within four wheat starch synthase proteins. The eight highly-conserved regions between the wheat starch synthase polypeptides are underlined and annotated at the top of each group of amino acid sequences. The sequences included in the alignment are the wheat SSII-A1 and wheat SSIII polypeptides of the present invention; wheat GBSS (wGBSS; SEQ ID NO:57 Yan et al., 1999); wheat SSI (wSS1; SEQ ID NO:58 Li et al., 1999); wheat SSII (wSS2; SEQ ID NO:4); and wheat SSIII (wSS3; SEQ ID NO:8).

On page 22, paragraph 1 (vii), lines 25-31, and page 23, paragraph 1 (viii), lines 1-13:

(vii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from a group consisting of:

- (A) KVGGLGDVVTS (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);
- (E) DVPIVGIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPCLTQLVAMRYGS (SEQ ID NO:45); and
- (H) TGGLVDTV (SEQ ID NO:46),

in addition to any one or more of (i) to (vi); and

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 6

(viii) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (A) KTGGLGDVAGA (SEQ ID NO:47);
- (B) GHRVMVVVPRY (SEQ ID NO:48);
- (C) NDWHTALLPVYLKAYY (SEQ ID NO:49);
- (D) GIVNGIDNMEWNPEVD (SEQ ID NO:50);
- (E) DVPLLGFIGRLDGQKG (SEQ ID NO:51);
- (F) DVQLVMLGTG (SEQ ID NO:52);
- (G) AGADALLMPSRF(E/V) PCGLNQLYAMAYGT (SEQ ID NO:53); and
- (H) VGG(V/L)RDTV (SEQ ID NO:54),

in addition to any one or more of (i) to (vi).

On page 24, paragraph 1, lines 2-9, and page 24, paragraph (vi), lines 13-21:

identity to an amino acid sequence selected from the group consisting of:

- (A) KVGGLGDVVTS (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);
- (E) DVPIVGIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPGLTQLVAMRYGS (SEQ ID NO:45); and
- (H) TGGLVDTV (SEQ ID NO:46).

in addition to any one or more of (i) to (iv); and

(vi) it which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (A) KTGGLGDVAGA (SEQ ID NO:47);
- (B) GHRVMVVVPRY (SEQ ID NO:48);

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 7

- (C) NDWHTALLPVYLKAYY (SEQ ID NO:49);
- (D) GIVNGIDNMEWNPEVD (SEQ ID NO:50);
- (E) DVPLLGFIGRLDGQKG (SEQ ID NO:51);
- (F) DVQLVMLGTG (SEQ ID NO:52);
- (G) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO:53); and
- (H) VGG(V/L)RDTV (SEQ ID NO:54),

in addition to any one or more of (I) to (iv).

On page 25, paragraph 2, lines 25-31:

Preferably, the isolated nucleic acid molecule of the present invention comprises a sequence of nucleotides which encodes, or is complementary to a nucleic acid molecule which encodes a wheat starch synthase polypeptide, protein or enzyme molecule or a functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS: 2, 4, 6, 8 or 10 and more preferably, which additionally comprises which comprises one or more amino acid sequences selected from the group consisting of:

- (A) KVGGLGDVVTS (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);
- (E) DVPIVGIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPGLTQLVAMRYGS (SEQ ID NO:45); and

On page 26, lines 1-9:

- (H) TGGLVDTV (SEQ ID NO:46);
- (I) KTGGLGDVAGA (SEQ ID NO:47);
- (J) GHRVMVVVPRY (SEQ ID NO:48);

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 8

- (K) NDWHTALLPVYLKAYY (SEQ ID NO:49);
- (L) GIVNGIDNMEWNPEVD (SEQ ID NO:50);
- (M) DVPLLGFIGRLDGQKG (SEQ ID NO:51);
- (N) DVQLVMLGTG (SEQ ID NO:52);
- (O) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO:53); and
- (P) VGG(V/L)RDTV (SEQ ID NO:54).

On page 28, paragraph 2 (iii), lines 28-31:

(iii) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (A) KVGGLGDVVT (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);

On page 29, lines 1-4 and paragraph (iv) lines 12-19:

- (E) DVPIVGIIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPGLTQLVAMRYGS (SEQ ID NO:45); and
- (H) TGGLVDTV (SEQ ID NO:46)

and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS:2, 4, 6, 8 or 10; and

(iv) a wheat starch synthase polypeptide, protein or enzyme or functional subunit thereof which comprises a conserved amino acid sequence having at least 25% identity to an amino acid sequence selected from the group consisting of:

- (A) KTGGLGDVAGA (SEQ ID NO:47);

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 9

- (B) GHRVMVVVPRY (SEQ ID NO:48);
- (C) NDWHTALLPVYLKAYY (SEQ ID NO:49);
- (D) GIVNGIDNMEWNPEVD (SEQ ID NO:50);
- (E) DVPLLGFIFGRDLGQKG (SEQ ID NO:51);
- (F) DVQLVMLGTG (SEQ ID NO:52);
- (G) AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO:53); and
- (H) VGG(V/L)RDTV (SEQ ID NO:54),

and wherein said wheat starch synthase polypeptide further comprises an amino acid sequence having at least about 85% identity overall to an amino acid sequence set forth in any one of SEQ ID NOS:2, 4, 6, 8 or 10.

On page 37, paragraph (iii), lines 13-20:

- (A) KVGGLGDVVTS (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);
- (E) DVPIVGIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPCLTQLVAMRYGS (SEQ ID NO:45);
- (H) TGGLVDTV (SEQ ID NO:46); (I) a wheat starch synthase ii (wSSII) polypeptide, protein or enzyme or functional subunit thereof which comprises an amino acid sequence which is at least about 85% identical overall to an amino acid sequence set forth in any one of SEQ ID NOS:2, 4 or 6;

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 10

On page 38, lines 3-18:

- (A) KVGGLGDVVT (SEQ ID NO:39);
- (B) GHTVEVILPKY (SEQ ID NO:40);
- (C) HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) GILNGIDPDIWDPYTD (SEQ ID NO:42);
- (E) DVPIVGIIITRLTAQKG (SEQ ID NO:43);
- (F) NGQVVLLGSA (SEQ ID NO:44);
- (G) AGSDFIIVPSIFEPCLTQLVAMRYGS (SEQ ID NO:45); and
- (H) TGGLVDTV (SEQ ID NO:46);
- (I) KTGGLGDVAGA (SEQ ID NO:47);
- (J) GHRVMVVVPRY (SEQ ID NO:48);
- (K) NDWHTALLPVYLKAYY (SEQ ID NO:49);
- (L) GIVNGIDNMEWNPEVD (SEQ ID NO:50);
- (M) DVPLLGFIGRLDGQKG (SEQ ID NO:51);
- (N) DVQLVMLGTG (SEQ ID NO:52);
- (O) AGADALLMPSTRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO:53); and
- (P) VGG(V/L)RDTV (SEQ ID NO:54).

On page 67, paragraph 3, lines 28-31:

Amino acid sequence comparisons between wheat BSSS, SSI, SSII and SSIII polypeptides reveals eight highly-conserved domains (Figure 9). The amino acid sequences of these domains are represented in the wheat SSIII amino acid sequence by the following sequence motifs:

- (A) Region 1: KVGGLGDVVT (SEQ ID NO:39);
- (B) Region 2: GHTVEVILPKY (SEQ ID NO:40);
- (C) Region 3: HDWSSAPVAWLYKEHY (SEQ ID NO:41);
- (D) Region 4: GILNGIDPDIWDPYTD (SEQ ID NO:42);

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 11

On page 68, lines 1-4 and paragraph 3, line 31:

(E) Region 5: DVPIVGIITRLTAQKG (SEQ ID NO:43);
(F) Region 5a: NGQVLLGSA (SEQ ID NO:44);
(G) Region 6: AGSDFIIVPSIFEPGLTQLVAMRYGS (SEQ ID NO:45); and
(H) Region 7: TGGLVDTV (SEQ ID NO:46).

For each of the amino acid sequences presented in the first column of Table 4, which are specific for wSSIII polypeptides, corresponding signature motifs which are specific for wSSII-A, wSSII-B, and wSSII-D polypeptides can be derived from the alignment, as follows:

Region 1: KTGGLGDVAGA (SEQ ID NO:47);

On page 69, lines 1-7:

Region 2: GHRVMVVVPRY (SEQ ID NO:48);
Region 3: NDWHTALLPVYLKAYY (SEQ ID NO:49);
Region 4: GIVNGIDNMEWNPEVD (SEQ ID NO:50);
Region 5: DVPLLGFGRDLGQKG (SEQ ID NO:51);
Region 5a: DVQLVMLGTG (SEQ ID NO:52);
Region 6: AGADALLMPSRF(E/V)PCGLNQLYAMAYGT (SEQ ID NO:53); and
Region 7: VGG(V/L)RDTV (SEQ ID NO:54).

On page 74, paragraph 2, lines 9-17, and paragraph 3, lines 19-25:

The catalytic domain of the starch synthases is found at the C-terminal end of the protein (Gao et al., 1998; Harn et al., 1998). Harn et al. (1998) identified 7 conserved regions among SSIIa, SSIIb, SSI and GBSS sequences. We have identified an

Applicants: Matthew Morrell et al.
Serial No.: 10/018,418
Filed : May 9, 2002
Page: 12

additional conserved region (designated region 5a in Table 4 and Figure 10) comprising the amino acid sequence motif DVQLVMLGTG (SEQ ID NO:52), by a comparison of the wSSII and wSSIII sequences of the present invention with differing isoforms of other plant starch synthase (GBSS, SS1, SSII and SSIII). The conservation of eight peptide regions among the 4 classes of starch synthases is striking, in terms of their sequence homologies and their alignment..

Analysis of the wheat SSII genes shows that there is a motif, PVNGENK (SEQ ID NO:59) which is repeated. The area surrounding the repeated PVNGENK (SEQ ID NO:59) motif is not homologous to maize SSIIa and the insertion of this region is responsible for the difference in length between the wheat SSII and maize SSIIa genes. In pea and potato SSII polypeptides, a PPP motif (Figure 3; residues 251-253 and 287-289 respectively) has been suggested to mark the end of the N-terminal region and to facilitate the flexibility of an "N-terminal arm". This motif is not found in either the maize or wheat SSII sequences.